Original Article

Periodontal health and relative quantity of subgingival *Porphyromonas gingivalis* during orthodontic treatment

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ABSTRACT

Objective: To examine periodontal changes and the relative quantity of subgingival *Porphyromonas gingivalis* during orthodontic treatment.

Materials and Methods: The study subjects were recruited consecutively among malocclusion patients seeking orthodontic treatment. Group A comprised 28 subjects (17.6 ± 5.68 years of age) at the beginning of orthodontic treatment, and group B comprised 20 subjects (17.8 ± 4.49 years of age) at the end of orthodontic treatment. Plaque index (Pl.I), gingival index (GI), and probing pocket depth (PPD) were measured before and after appliance placement in group A and before and after appliance removal in group B. Real-time quantitative polymerase chain reaction was used to quantify *P. gingivalis* in subgingival plaque at each time point.

Results: There was a significant increase in Pl.I and GI during the first 3 months of appliance placement but a significant decrease in Pl.I, GI, and PPD during the first 6 months after appliance removal. The carriage and relative quantity of subgingival *P. gingivalis* were high at the end of orthodontic treatment, and they decreased significantly after appliance removal. The amount of subgingival *P. gingivalis* after appliance removal (for a period of 6 months) was higher than the amount measured before appliance placement.

Conclusions: Fixed orthodontic treatment is conducive to dental plaque accumulation and gingival inflammation. In our study, after removal of orthodontic appliances, periodontal health improved, and the carriage and amount of subgingival *P. gingivalis* decreased. Nevertheless, the amount of subgingival *P. gingivalis* remained high for 6 months after appliance removal, and this finding might imply a potential risk to periodontal health in certain patients. (*Angle Orthod.* 2011;81:609–615.)

KEY WORDS: Fixed orthodontic treatment; Periodontal clinical examination; *Porphyromonas gingivalis*; Real-time PCR

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INTRODUCTION

Fixed orthodontic treatment is the preferred and most common method for treating malocclusion. Fixed appliances such as brackets, bands, or fixed retentions may complicate an optimal oral hygiene, and this may result in accumulation of dental plaque and gingival inflammation.[1,2] However, the effect of orthodontic treatment on the periodontal tissues in the long term is questionable. Bone loss may be observed during or immediately after termination of orthodontic treatment.[3] A 10-year retrospective study[4] concluded that orthodontic treatment during adolescence had no distinct effect upon later periodontal health.

Ample evidence indicates the gram-negative obligate anaerobe *Porphyromonas gingivalis* as a putative periodontal pathogen in subgingival dental plaque. *P. gingivalis* plays an important role in the onset and progression of periodontal diseases, and it is implicat-

ed as an indicator of periodontal disease.[5–9] Gingival changes and oral bacteria in plaque have been studied during orthodontic treatment in adolescents and young adults.[10–12] However, changes in periodontal health and the role of *P. gingivalis* in subgingival plaque at the different stages of orthodontic treatment have not received the same attention.

The aims of the present study were to examine changes in periodontal health in young subjects during orthodontic treatment and to assess the variation, if any, in subgingival *P. gingivalis* during the treatment.

MATERIALS AND METHODS

Participants

The participants were recruited consecutively among malocclusion patients seeking orthodontic treatment at the Orthodontics Section, First Affiliated Hospital of Dalian Medical University in China. Group A comprised 28 subjects, 22 females and 6 males (17.6 ± 5.68 years of age; range, 12 to 28 years), who were at the beginning of orthodontic treatment. Group B comprised 20 subjects, 13 females and 7 males (17.8 ± 4.49 years of age; range, 12 to 29 years), who were at the completion of orthodontic treatment. The inclusion criteria were the malocclusion angle's Class I, II, and III. The exclusion criteria were decayed teeth, bad oral habits (eg, thumb sucking, nail grinding, and mouth breathing), use of antibiotics or hormones 1 month before the study, concomitant systemic diseases, pregnancy, and lactation. Informed consent forms were signed by either the subjects themselves or their legal guardians.

Fixed Orthodontic Treatment and Oral Hygiene

All of the subjects were treated with straight-wire appliances on directly bonded brackets (Xinya, Hangzhou, China) on incisors, canines, and premolars and orthodontic bands cemented with glass-ionomer cement on the first molars. Professional oral cleaning and oral hygiene instructions on toothbrushing with the Bass technique and correct use of interdental cleaning devices were provided for all subjects 4 weeks before orthodontic treatment. After removal of the fixed appliances the patients were required to wear the Hawley removable retainer at night.

Periodontal Examination

A periodontal examination was performed by three calibrated examiners with a marked periodontal probe (YDM, Tokyo, Japan) at three time points for group A (Ta0 = just before appliance placement, Ta1 = 1 month after appliance placement, Ta2 = 3 months after appliance placement) and at four time points for group B (Tb0 = just before appliance removal, Tb1 = 1 month after appliance removal, Tb2 = 3 months after appliance removal, Tb3 = 6 months after appliance removal). Plaque index (PI.I),[13] gingival index (GI),[13] and probing pocket depth (PPD) were registered. Eight teeth from each subject (teeth 15, 13, 11, 21, 33, 31, 41, 45) were selected for periodontal examination, and each tooth was examined on its labial-mesial, labial-middle, and labial-distal sites. The score for each patient was the mean of the measurements on all of the teeth examined.

Subgingival Plaque Sampling

After gently removing supragingival plaque, subgingival plaque samples were taken by inserting a sterile dental curette into the bottom of the gingival crevice during the clinical examination. The samples were collected from the labial-medial and labial-distal surfaces of teeth 31, 32, 41, and 42. The samples from each tooth were pooled into an Eppendorf tube containing 1 mL of sterile saline and stored immediately at −70°C. *P. gingivalis* strain ATCC 33277 (Sichuan University) was used as a reference strain.

Quantification of *P. gingivalis* and Total Subgingival Bacteria by Real-Time Polymerase Chain Reaction

The subgingival plaque samples were thawed at room temperature and washed with Tris (pH 8.0). Total DNA was extracted by adding 10 mg/mL lysozyme; incubating in a 37°C water bath for 1 hour; adding 10% sodium dodecyl sulfate and 10 mg/mL proteinase K; incubating in a 56°C water bath for 12 hours; adding an equal volume of phenol/chloroform; and mixing well by inverting the tube until the phases were completely mixed. After centrifuging, the top layer was carefully transferred to a new tube. DNA was precipitated using 95% and 70% cold ethanol and was then dissolved in 20 μL TE buffer. DNA qualification was measured by electrophoresis and spectrophotometer analysis.

Real-time polymerase chain reaction (PCR) (TaqMan system) was applied to determine both the quantity of *P. gingivalis* and the quantity of the total bacteria cells presenting in subgingival plaque samples.[14,15] In order to eliminate the bias caused by sampling method, a relative quantity of *P. gingivalis* was used as *P. gingivalis* number per million total bacteria.[14] The primers and probes applied in real-time PCR were designed by TaKaRa Bio Inc, Dalian, China, (Table 1). PCR reaction was set up in a total volume of 25 μL containing Premix Ex Taq (2X) at 12.5 μL, each primer (10 μM) at 1 μL, probe (3 μM) at 1 μL, template DNA at 1 μL, and dH$_2$O at 8.5 μL. Real-time PCR ran at 95°C for 10 seconds; this was followed by 45 cycles

Table 1. Primers and Probes for Real-Time Polymerase Chain Reaction (PCR) Analyses[a]

Primers and Probes	Sequence	Product Size, Base Pairs	Reference
P. gingivalis 16S rDNA primer	Forward: 5′-TGT AGA TGA CTG ATG GTG AAA ACC-3′ Reverse: 5′-ACG TCA TCC ACA CCT TCC TC-3′	198	TaKaRa Bio Inc
P. gingivalis TaqMan probe	5′-(FAM) CGCCTTACGACGGCAGTCTCGGT (Eclipse)-3′		
Universal bacterial primer	Forward: 5′-CCA TGA AGT CGG AAT CGC TAG-3′ Reverse: 5′-GCT TGA CGG GCG GTG T-3′	85	
Universal bacterial TaqMan probe	5′-(FAM) -TAC AAG GCC CGG GAA CGT ATT CAC CG-(Eclipse)-3′		

[a] *P. gingivalis* indicates *Porphyromonas gingivalis*.

at 95°C for 5 seconds and 60°C for 30 seconds, with PCR Thermal Cycler Dice (TaKaRa Inc, Dalian, China).

Statistical Analysis

SPSS 16.0 for Windows was used for statistical analysis. Analysis of variance was used to analyze the longitudinal data of the periodontal indices and the square root of the relative quantity of subgingival *P. gingivalis* in each subject; an independent *t*-test was used for comparing the relative quantity of *P. gingivalis* between the two groups; and the Spearman test was used for correlation analysis. $P \leq .05$ was chosen as the significance level.

RESULTS

Clinical Findings

The measurements of Pl.I, GI, and PPD from two groups of subjects are shown in Table 2. Pl.I, GI, and PPD were low before the commencement of treatment. After the first 3 months of appliance wear, there was a significant increase in Pl.I and GI, but no obvious changes in PPD. By the end of the orthodontic treatment, Pl.I, GI, and PPD were rather high. After 3 to 6 months post–appliance removal, there was a significant decrease in Pl.I, GI, and PPD.

P. gingivalis in Subgingival Plaque

The frequency of patients with subgingival *P. gingivalis* during orthodontic treatment is shown in Figure 1. During the first 3 months of appliance wear,

the frequency with which the patient carried subgingival *P. gingivalis* decreased. The frequency of patients carrying subgingival *P. gingivalis* was high at the end of orthodontic treatment, and it dropped after appliance removal.

The relative quantity of subgingival *P. gingivalis* in patients following orthodontic treatment is shown in Figure 2. The relative amount of subgingival *P. gingivalis* varied largely among individuals. Generally, *P. gingivalis* was detected at a rather low proportion in the total subgingival bacteria during the beginning of orthodontic treatment. The relative amount of subgingival *P. gingivalis* was high at the end of orthodontic treatment, and this proportion decreased significantly 6 months after appliance removal. Nevertheless, the relative quantity of *P. gingivalis* after 6 months was higher than the quantity measured before appliance placement.

Association Between the Relative Quantity of *P. gingivalis* and Periodontal Conditions

In group A, no obvious association was found between the relative quantity of subgingival *P. gingivalis* and clinical periodontal indices. In group B, there was a significant correlation between the relative quantity of subgingival *P. gingivalis* and GI.

DISCUSSION

The present study has described periodontal and microbiological changes during fixed orthodontic treatment in two groups of patients. Group A was followed from the baseline until the 3 months after orthodontic

Table 2. Plaque Index (Pl.I), Gingival Index (GI), and Probing Pocket Depth (PPD) in Patients During Orthodontic Treatment[a]

	Group A (n = 28)			Group B (n = 20)			
	Ta0	Ta1	Ta2	Tb0	Tb1	Tb2	Tb3
Pl.I (Mean ± SD)	0.36 ± 0.45	0.66 ± 0.41*	0.87 ± 0.46*	0.99 ± 0.20	0.63 ± 0.26*	0.62 ± 0.29*	0.64 ± 0.24*
GI (Mean ± SD)	0.29 ± 0.54	0.96 ± 0.51*	0.96 ± 0.43*	1.7 ± 0.73	1.0 ± 0.56	0.5 ± 0.51*	0.45 ± 0.61*
PPD (Mean ± SD)	1.05 ± 0.11	1.17 ± 0.19	1.11 ± 0.22	1.68 ± 0.22	1.544 ± 0.24	1.49 ± 0.22*	1.41 ± 0.23*

[a] Ta0 indicates before orthodontic treatment; Ta1, 1 month after treatment start; Ta2, 3 months after treatment start; Tb0, before appliance removal; Tb1, 1 month after removal; Tb2, 3 months after removal; Tb3, 6 months after removal; and SD, standard deviation.
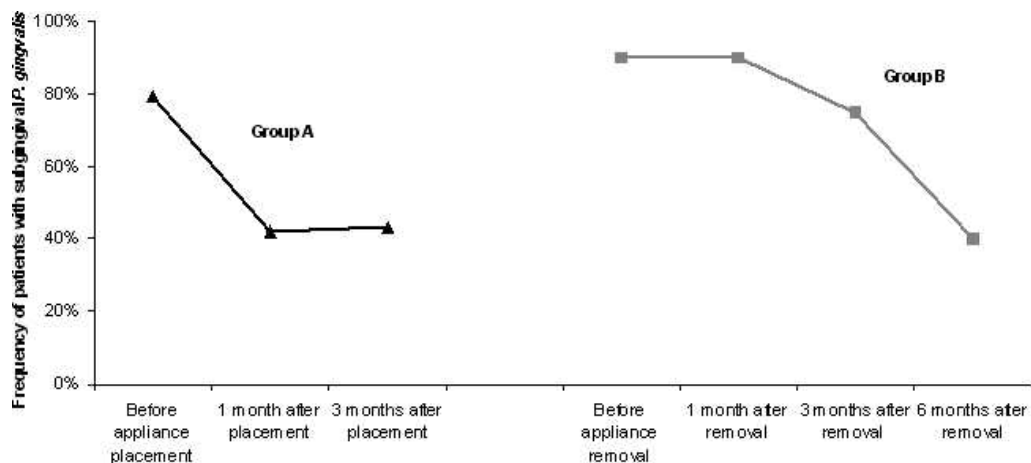
* $P < .05$.

Figure 1. Frequency of patients with subgingival *Porphyromonas gingivalis* during orthodontic treatment.

appliance placement. Group B was examined from just before removal of orthodontic appliance to 6 months postremoval. The two groups were comparable with respect to participants' age and gender.

Fixed orthodontic treatment resulted in dental plaque accumulation and gingival inflammation, with a significant increase in Pl.I and GI a short time after orthodontic treatment started (compared to the baseline). The treatment had no obvious effect on PPD. However, this observation might be affected by the short-term (3-month) evaluation period. Just before removal of the orthodontic appliance high scores of Pl.I, GI, and PPD were registered. The increase in plaque accumulation and gingival score during orthodontic treatment is probably a consequence of the plaque retentive effect of the orthodontic appliances, which makes performance of oral hygiene more difficult.[12]

A critical site for plaque accumulation is the excess composite around the bracket base (as a result of its rough surface and the presence of a distinct gap at the composite enamel surface).[16] The placement of brackets should therefore be done under optimal conditions. Six months after orthodontic appliance removal, Pl.I, GI, and PPD decreased significantly among the patients in group B. The orthodontic treatment seemed to have no permanent effects on the oral hygiene situation and gingival inflammation. One must keep in mind that some subjects in this study were in the circum-pubertal age range. It has been shown[17] that gingival inflammation and gingival bleeding will increase in children of pubertal age as a result of the hormone changes that occur during puberty. In the literature the influence of orthodontic treatment on periodontal tissues is somewhat unclear. A systematic review on the effect of orthodontic therapy on
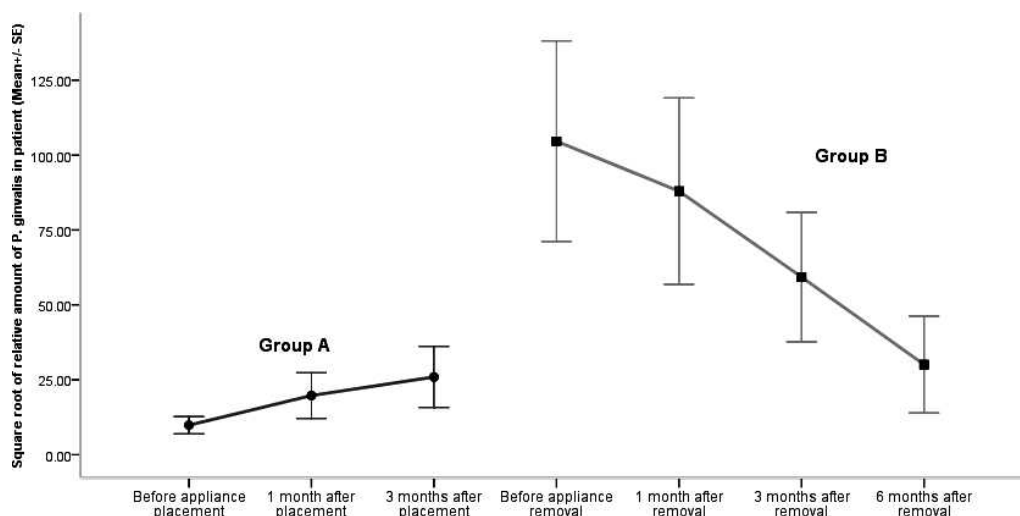

Figure 2. Relative quantity of subgingival *Porphyromonas gingivalis* in patients during orthodontic treatment.

periodontal health concluded that the effects of orthodontic therapy on gingivitis and attachment loss were inconsistent across studies and that there is an absence of evidence describing positive effects of orthodontic treatment on periodontal health, but many findings indicate that orthodontic therapy may result in small detrimental effects to the periodontium.[2] Orthodontic treatment followed by the use of postorthodontic fixed retainers has been found[18] to be associated with an increased incidence of gingival recession, increased plaque retention, and increased bleeding on probing. The magnitude of the recession was of low clinical significance.

The subjects in the present study were supplied with a removable retention appliance, and this may be one of the reasons for the better oral hygiene and gingival conditions found. Gingival recession was not registered in our study. Some studies have shown that fixed orthodontic treatment may result in periodontal bone loss within a short period after the placement of bands and brackets[12,19,20] and loss of clinical attachment after fixed orthodontic treatment.[12,21] In a longitudinal study of alveolar bone loss in orthodontic-treated teenagers over a 4-year period, Aass and Gjermo[20] found that the majority of the bone loss observed during or immediately after the termination of orthodontic treatment was transient in nature. In a follow-up study conducted 12–35 years after comprehensive fixed appliance orthodontic treatment during adolescence, Sadowsky and BeGole[22] demonstrated that orthodontic treatment in adolescence is not a major factor in determining long-term periodontal health status. They found that no significant amount of either damage or benefit to the periodontal structures could be directly attributed to orthodontic therapy. A controlled clinical study[4] of persons who had completed orthodontic therapy at least 10 years previously compared to a group of adults with untreated malocclusion demonstrated that orthodontic treatment during adolescence had no distinct effect upon later periodontal health.

P. gingivalis is a member of the normal microbiota of the oral cavity, which comprise more than 700 different species of microorganisms. *P. gingivalis* has been detected in 25% of the healthy subjects and in 79% of a periodontitis group by a specific PCR assay.[5] However, isolation of this oral anaerobe has proven difficult in a healthy oral cavity.[23] *P. gingivalis* has been suggested[27] as a possible etiologic agent for periodontal lesions, infections, and adult periodontal diseases[24–26] through its production of a number of virulence factors (eg, collagenase, hemolysins, proteases, endotoxin, and fatty acids). In the present study, real-time PCR was the technique used to identify and quantify *P. gingivalis* in subgingival plaque. Real-time

PCR is a relatively new molecular technique used to assay a small amount of template DNA from periodontal pathogens. It has been used for identification of *P. gingivalis* and represents a sensitive, efficient, and reliable approach in *P. gingivalis* detection.[28–30]

The present study showed that the frequency of subjects with subgingival *P. gingivalis* decreased during the first 3 months after placement of orthodontic appliances. This finding agrees with that of another study[31] that found a statistically significant decrease in the number of patients with *P. gingivalis* and *P. nucleatum* in plaque samples taken up to 6 months after application of fixed appliances. Despite an increase in Pl.I and GI, the relative quantity of subgingival *P. gingivalis* remained stable after subjects wore the orthodontic appliances. The explanation may be that the placement of fixed appliances initially disrupts supra- and subgingival dental plaque that causes subgingival plaque in a new dynamic state, where aerobic bacteria start to boost and anaerobes reduce. This disruption may have a long-lasting effect on plaque composition.[32] Speer et al.[33] attributed the reduction in subgingival aerobes and anaerobes under fixed appliance therapy to metal corrosion and the release of primarily nickel ions, which have a toxic effect on bacteria.

At the completion of the orthodontic treatment both the frequency of *P. gingivalis* carrier and the amount of subgingival *P. gingivalis* were high. The increasing *P. gingivalis* could be the result of abundant dental plaque on the tooth surface and increasing gingival inflammation. In this episode, the booming facultative and aerobic microorganisms consume oxygen and provide nutrients, enzymes, and coadhesions that are favorable to the colonization of anaerobic bacteria.[34] Gingival inflammation enhances the flowing of gingival crevicular fluids that supply plasma proteins, such as albumin, lactoferrin, and lysozyme, which are essential for the growth of proteolytic anaerobes.[34] It has been shown[7,34] that the microflora associated with gingivitis is diverse with *Actinomyces species*, capnophilic, and obligate anaerobic gram-negative bacteria being predominant. After removal of the orthodontic appliance, both *P. gingivalis* carriage and the relative amount of subgingival *P. gingivalis* were reduced significantly in 6 months. This reduction correlated with the decrease of gingival inflammation, and such correspondence was also found in other studies[25,35] that showed a significant reduction in *P. gingivalis* following an improvement in periodontal health. Kawada et al.[25] reported a positive correlation between the number of *P. gingivalis* and pocket depth, while others have shown[5,9,36] that *P. gingivalis* occurred with greater frequency and at higher levels at sites of disease activity.

Despite a significant decrease in the relative quantity of subgingival *P. gingivalis* after appliance removal, the *P. gingivalis* quantity 6 months after appliance removal was still higher than the quantity measured before appliance placement. This indicates that there might be a potential risk to periodontal health from orthodontic therapy in certain people, which needs to be further verified by a long-term prospective study on one group of subjects.

CONCLUSIONS

- Fixed orthodontic treatment is conducive to dental plaque accumulation and gingival inflammation. Accordingly, after removal of the orthodontic appliances, the periodontal condition improved.
- The carriage and the amount of *P. gingivalis* in subgingival plaque were high at the end of orthodontic treatment, while they decreased after orthodontic appliance removal. Nevertheless, the quantity of subgingival *P. gingivalis* remained high at the end of the treatment compared to the quantity measured before treatment.

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